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Structural abnormality of the hippocampus associated with depressive symptoms in heart failure rats

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ABSTRACT

Heart failure (HF) is characterized by a blood supply which is insufficient to meet the body's demand. HF can potentially affect the brain and is associated with a high prevalence of depression. However, the mechanisms by which the two are related remain largely unclear. Structural abnormalities of the ventral hippocampus have been observed in depression but have never been reported in HF. In this study, we thus investigated structural brain abnormality in HF using voxel-based morphometry (VBM) and histological analysis in a rat model of HF. T2-weighted images were obtained in rats with HF (n = 20) and sham rats (n = 17) and VBM was used to produce gray matter concentration (GMC) maps. Twenty-four hour locomotor activity was used as a sign of depressive behavior. Brains of HF and sham rats (n = 8, each) were fixed and histologically analyzed for the measurement of neurogenesis, the number of astrocytes and neurite outgrowth in the ventral hippocampus. VBM demonstrated significant GMC decrease in the hippocampus, which was restricted to the ventral segment. Similarly, neurogenesis and neurite outgrowth were significantly decreased and the number of astrocytes was significantly increased in HF rats as compared with sham rats in the ventral hippocampus. GMC values in the ventral hippocampus were significantly and negatively correlated with 24 hour locomotor activity in HF rats. In conclusion, the present study has demonstrated for the first time that the structural abnormality of the ventral hippocampus is associated with depressive symptoms in HF rats.

Introduction

Heart failure (HF) is characterized by insufficient blood supply for the body demand due to cardiac dysfunction and often occurs as a result of myocardial ischemia. Low cardiac output and chronic stress in HF can potentially affect the brain. Prevalence of depression is increased among HF patients (Rutledge et al., 2006), which suggests that there may also be coexisting structural brain abnormalities. Depression complicated with HF is associated with increased risk of mortality and rehospitalization in patients with HF (Jiang et al., 2001; Rutledge et al., 2006) and thus should be investigated to improve outcomes. However, the mechanism of depression in HF remains largely unclear.

The hippocampus is an important brain center, which is involved in emotion and memory, and can be subdivided into the ventral and dorsal segments. Lesioning the ventral and dorsal hippocampi in rodents can lead to aberrant expression of anxiety and memory deficits, respectively (Kjelstrup et al., 2002; Broadbent et al., 2004; Fanselow and Dong, 2010). Behaviorally depressed cynomolgus macaques showed reduced volume of the anterior hippocampus (Willard et al., 2009), which is analogous to the ventral hippocampus in rodents. The ventral hippocampus is more vulnerable to decreased neurogenesis than the dorsal hippocampus in rodent models of depression-like behavior (Tanti and Belzung, 2013). To the best of our knowledge, however, there is no definite evidence that structural abnormalities of the ventral hippocampus have been previously observed in HF.

Voxel-based morphometry (VBM), an unbiased technique based on magnetic resonance imaging (MRI), has been used to assess regional differences in the concentration or volume of a particular tissue such as brain gray matter (Ashburner and Friston, 2000, 2001; Quallo et al., 2009; Sawiak et al., 2009; Yang et al., 2011; Biedermann et al., 2012; Suzuki et al., 2013b). There have already been three VBM studies recruiting HF patients (Woo et al., 2003; Menteer et al., 2010; Almeida et al., 2012), which did not consistently reveal structural abnormalities of the hippocampus. Probably this is because patients with HF usually have many confounding factors affecting gray matter such as

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hypertension, diabetes, dyslipidemia, smoking and administration of antihypertensive drugs (Ward et al., 2010; Zhang et al., 2011; Glodzik et al., 2012; Moran et al., 2013). Therefore, structural brain abnormalities in HF should be assessed using experimental animals that have uniform environmental and genetic backgrounds. We previously reported an in-vivo rat T2 MRI template that includes three different classes of tissue probability maps (Valdés-Hernández et al., 2011). VBM using our rat template could successfully detect structural abnormalities of the hippocampus in a rat model of cardiopulmonary resuscitation (Suzuki et al., 2013b).

In the present study, we assessed structural brain abnormality in HF using VBM and histological analysis in a rat model of HF. We examined changes in neurogenesis, the number of astrocytes and neurite outgrowth in the ventral and dorsal hippocampi histologically. Twenty-four hour locomotor activity, an index of depressive symptoms in rodents (Fukamauchi et al., 1996; Kabuki et al., 2009), was also measured in both HF and sham rats before MRI recordings. Increased locomotor activity in 24 hour spontaneous measurement is observed in rodent models of depression or chronic stress (Fukamauchi et al., 1996; Kabuki et al., 2009). The purpose of the present study was to investigate the following four hypotheses: (1) VBM detects gray matter changes in the ventral hippocampus in HF rats, (2) neurogenesis, the number of astrocytes and neurite outgrowth are changed in the ventral hippocampus in HF rats, (3) 24 hour locomotor activity is increased in HF rats, and (4) VBM-detected gray matter changes in HF rats are associated with depressed symptoms examined with 24 hour locomotor activity measurements.

Materials and methods

Animals

A total of 53 male Wistar rats (9 week-old; Charles River, Japan) were assigned to either HF group (n = 28) or sham group (n = 25). The HF and sham rats were used either for MRI recordings followed by behavioral tests (20 HF and 17 sham rats) or histological analysis (8 HF and 8 sham rats). All procedures and protocols were performed in accordance with the policies established by the Animal Care Committee at Tohoku University, Sendai, Japan (approval number: 2012-241).

The HF rats

The coronary ligation protocol was performed as previously described (Henderson et al., 2009). Briefly, rats were anesthetized with isoflurane, and were orally intubated for mechanical ventilation using a ventilator (Harvard Apparatus, Holliston, MA, USA). The following surgical preparations were performed in the prone position on a hotplate (AS ONE, Osaka, Japan) under 1.5% isoflurane. The chest was opened at the 4th intercostal space to expose the heart, the pericardium was incised and the left anterior descending coronary artery was ligated between the pulmonary outflow tract and the left atrium with a 6-0 silk suture. The lungs were re-inflated by momentarily occluding the outflow of the ventilator. The chest was closed with 3-0 silk sutures. Weight-matched rats were used as controls and underwent all surgical steps except for the coronary ligation (sham rat). The HF and sham rats were returned to their cages where they were housed for 16 weeks in a room on a 12-hr light–dark cycle until the time of the behavioral studies or histological analysis.

Behavioral tests

Sixteen weeks after the coronary ligation or the sham operation protocol, 24 hour locomotor activity measurement was performed as described in our previous study (Okuda et al., 2004). After adaptation for three days, 24 hour locomotor activity was measured using an infrared ray sensor system (SUPER-MEX, Muromachi-Kikai, Tokyo, Japan) that consists of twelve small compartments divided with walls on a large shelf. The size of each compartment is 40 cm wide × 50 cm long × 35 cm high, and each compartment is equipped with a ceiling sensor that can detect heat energy radiated from the rats. The system monitors rat movement by measuring changes in heat energy in the covered field. Rats were placed individually in the compartments within steel wire cages.

Echocardiographic assessment

In vivo heart function and structure were assessed by echocardiography using a Vevo 2100 system (VisualSonics, Toronto, Ontario, Canada) designed specifically for small animal studies as previously described (Morgan et al., 2004). The echocardiographic assessment was performed before MRI recording or histological analysis and within 4 days of the 24 hour locomotor activity measurement. Rats were anesthetized with 2% isoflurane administered via a nose cone, were shaved from the chest and were placed in the supine position on a hotplate (AS ONE, Osaka, Japan). Two-dimensional (2D) and M-mode echocardiography images were obtained from the parasternal short-axis view to measure fractional shortening (FS), interventricular septal thickness (IVSTd), left ventricular diastolic dimension (LVDd), left ventricular systolic dimension (LVDs) and posterior wall thickness in diastole (PWTd) (Fig. 1). FS was calculated as percentage in accordance with the following formula: FS = (LVDd − LVDs) / LVDd × 100(%). All measurements were carried out offline using the Vevo 2100 system software and were averaged from three cardiac cycles.

MRI recording

MRI recording was performed as described in our previous studies (Sumiyoshi et al., 2012; Suzuki et al., 2013a,b). Briefly, rats were initially anesthetized with isoflurane, and polyethylene catheters were inserted into the femoral artery and vein to examine blood pressure and systemic drug delivery, respectively. The rats were orally intubated for artificial ventilation, were placed in the prone position on a custom-built MRI bed with a bite bar, and mechanically ventilated at a respiration rate of 60 ± 1 breaths/min using a ventilator (SAR-830/AP, CWE Inc., Ardmore, PA, USA). After the rats received a bolus injection of pancuronium (2 mg/kg), anesthesia was maintained at 1% isoflurane with continuous administration of pancuronium (2 mg/kg/h).

All MRI data were acquired using a 7.0 T Bruker PharmaScan system (Bruker Biospin, Ettlingen, Germany) with a 38-mm-diameter bird-cage coil. Prior to all MRI acquisitions, we first performed global magnetic field shimming inside the core and subsequently localized on the region of interest (ROI) using a point resolved spectroscopy protocol. The linewidth (full width at half maximum) at the end of the shimming procedure ranged from 10 to 16 Hz in the ROI (approximately 300 μl). T2-weighted images (T2WIs) were obtained using a 2D-RARE sequence with the following parameters: TR = 4600 ms, TEeff = 30 ms, RARE factor = 4, SBW = 100 kHz, flip angle = 90°, FOV = 32 × 32 mm^2, matrix size = 256 × 256, voxel size = 125 × 125 μm^2, number of slices = 54, slice thickness = 0.5 mm, slice gap = 0 mm, and number of repetitions = 10.

Measurement of cardiac infarct size

The measurement of infarct size protocol was performed as modified in the past study (Saeed et al., 2001). At the end of each MRI recordings, the heart was removed and was cross-sectioned from base to apex into four short-axis slices. The slices were weighed to measure heart weight and were immersed in distilled water that contained 1.0% triphenyltetrazolium chloride (TTC; Sigma Aldrich; Munich, Germany) for 20 min at 37 °C to identify infarcted tissue. Infarct size was calculated as mean value of infarct circumference divided by total